Development of Species-Specific SCAR Markers in Bentgrass

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ABSTRACT

Bentgrass species (*Agrostis* spp.) are cool season turfgrasses that are tolerant of continuous, close mowing heights because of their prostrate growth habit. Some bentgrass species are difficult to distinguish because of similar morphological characteristics and genetic compatibility. Specific DNA technology, such as the use of SCAR (sequence characterized amplified region) markers, can be used to differentiate between some species of bentgrass. SCAR markers were created by sequencing a single RAPD (random amplified polymorphic DNA) band and designing primers to amplify the band of specific size. Two SCAR primer pairs were designed to identify colonial (*Agrostis capillaris* L.) and creeping (*Agrostis palustris* Huds.) bentgrass species. The colonial SCAR primers amplify a band of 400 base pairs (bp) and are designated Col400F and Col400R. The creeping SCAR primers amplify a band of 700 bp and are designated Creep700F and Creep700R. Testing with 17 cultivars (140 plants) representing four bentgrass species, single unique bands were correctly amplified for creeping and colonial bentgrass species by their respective SCAR primer pair. Differentiating these species by SCAR markers is useful for screening large numbers of clones collected from superior patches of naturalized populations on old golf courses and lawns. These SCAR markers also have useful potential for identifying progenies derived from artificial interspecific hybridizations among bentgrass species, especially between colonial and creeping bentgrass.

Bentgrass species are cool season turfgrasses native to western Europe. There are over 200 species of bentgrass with five species used commercially in the USA (Hitchcock, 1951). These include creeping bentgrass (*A. palustris*), colonial bentgrass (*A. capillaris*, syn = *A. tenuis* Sibth.), velvet bentgrass (*A. canina* L.), and redtop bentgrass (*A. alba* L.). Another bentgrass species called dryland (*A. castellana* Boiss. and Reuter) is also used; however, it is often mistakenly grouped with colonial bentgrass (Brilman, 2001a). Creeping and colonial bentgrass are the two most commonly used species. Creeping bentgrass is generally used for golf course putting greens, tees, and closely mowed fairways because of its tolerance of low mowing heights and its strong stoloniferous growth habit (Beard, 1973). Colonial bentgrass is used on golf courses, lawns, and in erosion control (Beard, 1973; Hitchcock, 1951). However, it is often considered inferior to creeping bentgrass for greens and fine turfgrass areas because some varieties have an unfavorable growth habit (Dudeck and Duich, 1967). Colonial bentgrass is known to be susceptible to diseases such as brown patch (caused by *Rhizoctonia solani* Kühn) and red thread [caused by *Laetisaria fuciformis* (McAlpine) Burdsall] (Beard, 1973; Brilman, 2001a). However, some cultivars of colonial bentgrass are beginning to show improved resistance to brown patch (Brilman, 2001a; Murphy et al., 2000) and dollar spot (caused by *Sclerotinia homoeocarpa* F.T. Bennett), with variation of disease severity existing among cultivars (Meyer and Bonos, 2001; Murphy et al., 2000). Hybrids of the two bentgrass species show increased disease resistance and better turf quality (Brilman, 2001a). With more and more restrictions on fungicide use, it is becoming important to improve creeping bentgrass cultivars resistant to turfgrass diseases.

Because of the complexity of the *Agrostis* genus, creeping and colonial bentgrass species are very difficult to classify solely on the basis of their morphological characteristics (Yamamoto and Duich, 1994). Both are characterized by dense mats, strong stoloniferous growth, shallow roots, long narrow leaves, and a light green to bluish green appearance (Beard, 1973). Another complication in identification results from natural interspecific hybridization between creeping and colonial bentgrass and artificial interspecific hybridization forced by breeders (Stuckey and Banfield, 1946; Bradshaw, 1958). Because colonial and creeping bentgrass are genetically compatible, natural hybridization occurs, creating a variety of clones with mixed traits. Currently, identification of hybrids relies on morphological characters such as various flowering structures. Natural hybrids of creeping and colonial bentgrass that survive biotic and abiotic stresses can be mistaken for a true colonial or creeping bentgrass species. Thus, clones collected from old turf could easily be mislabeled. This has already occurred in New York where creeping and colonial bentgrass hybrids taken from a golf course were mistakenly labeled as colonial bentgrass because of their growth form (Brilman, 2001b). On the other hand, breeders intentionally hybridize creeping and colonial bentgrass species to combine important traits, such as brown patch resistance, into a single clone. It is necessary to identify which of these offspring are true hybrids. This is particularly difficult in creeping and colonial bentgrass hybrids where there are no major morphological traits differentiating parents. Therefore, it would be best to use a molecular test coupled with morphological characteristics to detect hybrids.

Several molecular methods have been used to distinguish between bentgrass species and cultivars. Acrylamide gel disk electrophoresis was used by Wilkinson and Beard (1972) to distinguish seed and vegetatively propagated creeping bentgrass varieties. Isozyme elec-

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Published in Crop Sci. 43:345–349 (2003).

Abbreviations: bp, base pairs; RAPD, random amplified polymorphic DNA; RFLP, restriction fragment length polymorphism; SCAR, sequence characterized amplified region.

trophoresis methods to identify creeping bentgrass cultivars were utilized by Warnke et al. (1997). However, Jones (1983) reported isozyme instability after storage, resulting in different banding patterns. Another drawback of isozyme analysis is the limited number of markers available (Golembiewski et al., 1997). More recent and robust methods use DNA markers as tools in mapping important traits and fingerprinting cultivar and species differences. Restriction fragment length polymorphic (RFLP) markers have been used to distinguish some creeping bentgrass cultivars, but only a limited number of cultivar-specific markers have been identified (Caceres et al., 2000). RAPD is another identification tool. On the basis of PCR (polymerase chain reaction), RAPD has the advantage of being rapid, cost effective, and has been used for identification of crops such as buffalograss (*Buchloë dactyloides,* Nutt.), Kentucky bluegrass (*Poa pratensis* L.), and bentgrass (Huff et al., 1993; Huff, 2001; Golembiewski et al., 1997). However, the technique can be difficult to reproduce because of its high sensitivity to reaction conditions (Skroch and Nienhuis, 1995).

More recently, SCAR DNA analysis was developed to produce more specific and reproducible results (Paran and Michelmore, 1993; Jung et al., 1999). SCAR markers are created by using a longer primer (extended sequence of a RAPD primer) that has a specific sequence of approximately 20 bases. By increasing the specificity, the results are less sensitive to changes in reaction conditions and are more reproducible (Hernandez et al., 1999). Reliable SCAR markers have already been successfully derived from RAPD fragments in *Lettuca*, *Vicia*, and *Triticum* (Paran and Michelmore, 1993; Vidal et al., 2000; Hernandez et al., 1999). The objective of this study was to design robust genetic markers that distinguish creeping and colonial bentgrass species by SCAR technology. These SCAR markers will be useful for screening clones collected from naturalized populations and have potential for identifying creeping and colonial bentgrass progenies derived from interspecific hybridizations.

MATERIALS AND METHODS

Germplasm

Seventeen cultivars of creeping, colonial, velvet, and dryland bentgrass species were used to design species-specific SCAR primers (Table 1). The samples were obtained from Olds Seed Co. in Wisconsin. Up to 10 plants of each cultivar were used. Each plant was grown from a single seed, planted in a single conical (2.5-cm diam by 16.5-cm height) container and maintained under greenhouse conditions where soil was kept moist. Each plant was fertilized with 2 g/L 20N:20P:20K soluble fertilizer biweekly. Any additional plants growing in the container were removed to ensure each genotype (plant) came from a single seed for DNA extraction.

DNA Extraction

The DNA extraction followed Johns et al. (1997) protocol with the minor modification in the method for grinding tissue sample tissues. DNA was extracted from 0.1 to 0.2 g of fresh sample tissue. The tissue was ground with 500 μL potassium ethyl xanthogenate modified extraction buffer in FastPrep FP120 machine (BIO 101 Inc., Carlsbad, CA) using a ceramic bead. The samples were then heated in a 65°C water bath for 30 min. The samples were centrifuged at 8161 *g* for 10 min and the supernatants were transferred to a clean 1.5-mL microcentrifuge tube. Nucleic acids were precipitated by means of 6:1 95% (v/v) ethanol and 7.5 *M* ammonium acetate for 30 min at room temperature. The nucleic acids were then pelleted at 1306 *g* for 10 min. Tris EDTA+RNase was added to the pellet and incubated at 37°C for 1 h. Next, plant debris was pelleted, and the supernatant was transferred to a clean 1.5-mL tube and the DNA was precipitated from the supernatant with 10:1 95% ethanol and 3 *M* sodium acetate for 30 min at room temperature. The samples were spun at 2040 g for 5 min to pellet the DNA. The DNA was washed with ethanol and the DNA pellets were collected through centrifugation. Finally, the DNA was quantified with a DNA Fluorometer Model TKO-100 (Hoefer Scientific Instruments), then diluted to 4 μ*M* in Tartrazine + 0.1× Tris EDTA for use in PCR reactions.

Table 1. List of bentgrass cultivars used to design species-specific SCAR primers including five colonial (*A. capillaris*) cultivars, seven creeping (*A. palustris*) cultivars, three velvet (*A. canina*) cultivars, and two dryland (*A. castellana*) cultivars.

Cultivar	Species	Common name	Number of plants
Astoria	*Agrostis capillaris*	Colonial	10
Bardot	*Agrostis capillaris*	Colonial	10
SR7100	*Agrostis capillaris*	Colonial	10
9F7	*Agrostis capillaris*	Colonial	6
Tiger	*Agrostis capillaris*	Colonial	10
Providence	*Agrostis palustris*	Creeping	7
Penncross	*Agrostis palustris*	Creeping	10
Cato	*Agrostis palustris*	Creeping	10
Penn G1	*Agrostis palustris*	Creeping	6
Penneagle	*Agrostis palustris*	Creeping	10
Seaside II	*Agrostis palustris*	Creeping	10
SR1119	*Agrostis palustris*	Creeping	10
EVM 99	*Agrostis canina*	Velvet	6
SR7200	*Agrostis canina*	Velvet	5
Kingstown	*Agrostis canina*	Velvet	5
Highland	*Agrostis castellana*	Dryland	10
Exeter	*Agrostis castellana*	Dryland	5

Purification for Sequencing

Thirteen RAPD primers (Operon Technologies Inc., Alameda, CA) were previously screened on 96 bentgrass clones collected from old golf courses in Wisconsin (Wang et al., 2000). Two RAPD primers, AF6 and P8, were chosen for their consistent banding patterns that amplified many different-sized, bright fragments between 200 to 1600 bp. The RAPD reactions were performed in 10-μL volumes in 96 well plates in an MJ PTC-100 (MJ Research, Watertown, MA) with thermal cycling conditions as described by Johns et al. (1997) of 91°C for denaturation, 42°C for annealing, and 72°C for elongation. Forty cycles were performed with the first cycle timing of 60 s for denaturation, 15 s for annealing and 70 s for elongation. Subsequent cycles were the same except for only a 15-s denaturation period. PCR products were run in a 1.5% (w/v) agarose gel and stained in distilled water containing 0.5 μg/mL ethidium bromide for 20 to 30 min and destained in distilled water for 20 to 30 min. A bright and highly reproducible RAPD band was chosen that was only amplified in a single species.

To convert the selected RAPD band to a SCAR marker, the bands were excised, cloned, and sequenced following the procedure outlined in Jung et al. (1999). Two excised PCR bands were sequenced at the University of Wisconsin Biotech-

nology Center DNA Sequencing facility (Madison, WI). For each PCR band, two white colonies were chosen after transformation for sequencing to ensure that the PCR product selected for sequencing was predominant. The SCAR marker sequences were designed by identifying the original 10-bp sequence of the RAPD primer and adding the next approximately 10 bp in the DNA sequence. These species-specific SCAR primers were synthesized by Operon Technologies Inc.

Analyzing SCAR Primers on Bentgrass DNAs

Each of the designed SCAR primer pairs (one forward and one reverse SCAR primer) was tested by means of a subsample of three cultivars: one each of creeping, colonial, and velvet bentgrass species (Cato, Bardot, EVM99, respectively). Testing was done to ensure amplification of the band with exact molecular weight and determine optimal annealing temperature. Three different annealing temperatures (55, 62, and 65°C) were screened to determine the optimal annealing temperature. Conditions of SCAR reactions were the same as RAPD reactions described by Johns et al. (1997) with the exception of changes to the annealing temperature. Once the optimal annealing temperature for each set of SCAR primer pairs was determined, SCAR primers were run on all 140 bentgrass plants (Table 1) to ensure the SCAR primer pairs correctly identified their respective bentgrass species. PCR products were run on a 1.5% (w/v) agarose gel and stained in ethidium bromide as stated above. Presence and absence of the SCAR band was visually scored and compared with samples of each species.

RESULTS AND DISCUSSION

Selecting RAPD Bands for Conversion

One hundred-forty plants consisting of 17 cultivars (four bentgrass species) (Table 1) were used to develop species-specific SCAR markers. On the basis of morphological traits, such as stoloniferous growth habit, along with RAPD banding patterns that are consistent within species, we determined all plants used in this experiment were bentgrass. Some bentgrass cultivars had less than 10 plants because of poor germination of seeds. Once DNAs were collected, we used RAPD screening to find a bright and consistent band that was specific to a particular bentgrass species. In a previous study, we ran 13 RAPD primers on 96 bentgrass clones collected in Wisconsin (Wang et al., 2000). From this data, two primers, AF6 and P8, which amplified a high number of bands consistently among selected plants, were chosen. Subsequently, the two RAPD primers were tested on the DNA of 140 bentgrass plants (Table 1). One bright and reproducible band, approximately 700 bp in size, was amplified with the RAPD primer AF6 only in creeping bentgrass species (Fig. 1a). Using the RAPD primer P8, we amplified a bright and distinct band, approximately 400 bp, in both colonial and dryland bentgrass species (Fig. 1b). Both bands, indicated by arrows, were selected for conversion into species-specific SCAR markers (Fig. 1).

Cloning Selected RAPD Bands

To ensure that the most representative PCR product, out of a pool of heterogeneous RAPD amplified products, was selected for sequencing, two transformed white colonies from each excised RAPD band were selected for sequencing. The sequences of both colonies were compared for each sample, and no differences were observed. It was also noted that the first 10 bp of the sequence corresponded to the appropriate RAPD primer used to find a distinguishing band for each species. To design the species-specific SCAR marker pairs, these first 10 bp were used plus the next nine to 10 bases in the sequence. In Table 2, the sequences of the designed primers are listed with the RAPD primer sequence underlined. The SCAR primer pair Creep700F/Creep-700R was named because of amplification in the creeping bentgrass at approximately 700 bp. Similarly the colonial primers were named Col400F/Col400R because of the amplification of a band at approximately 400 bp. The primer pairs were first screened on three DNAs of bentgrass (one each of creeping, colonial and velvet species) to determine the optimal annealing temperature of 65°C (Table 2).

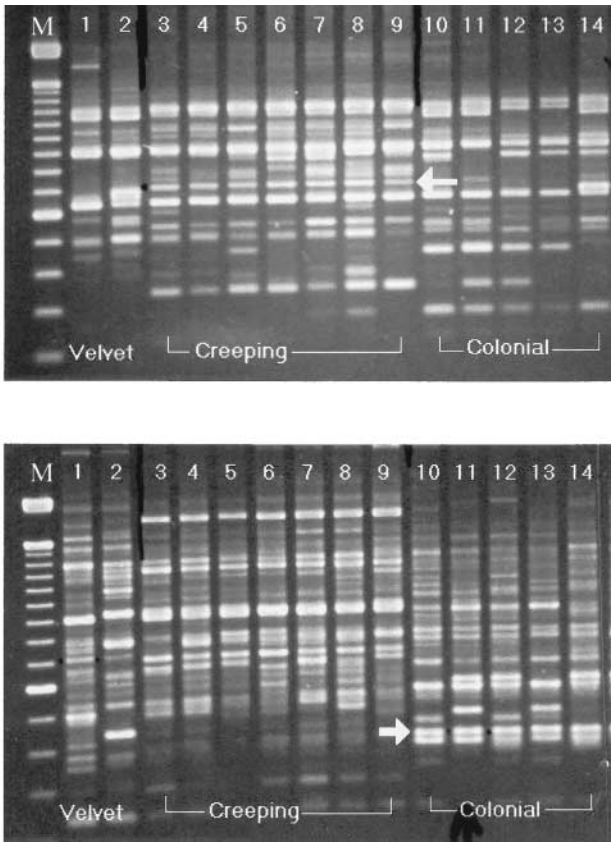

Fig. 1. RAPD banding patterns of Operon primers AF6(a) and P8(b) and 14 bentgrass DNAs. Arrows indicate species-specific, strong and reproducible RAPD bands that were amplified in creeping(a) and colonial(b) bentgrass species and converted into species-specific SCAR markers. Bentgrass plants indicated are two velvet bentgrass plants of cultivar EVM 99 (Lanes 1 and 2), seven creeping bentgrass plants of Cato (Lanes 3–9), and five colonial bentgrass plants of '9F7' (Lanes 10–14). "M" indicates 100-bp marker with a bright middle band estimating 600 bp.

Testing Designed SCAR Primers

The SCAR primers were then used to amplify all 140 bentgrass DNAs. A single, distinct, and easily identifi-

Table 2. Bentgrass species-specific SCAR primer pair sequences derived from cloned RAPD fragments. Optimal annealing temperatures for each set of SCAR primers are noted.

RAPD primer	SCAR primer†	5′ to 3′ Sequence†	Annealing temperature
AF6	Creep700F	CCGCAGTCTGGGCTAACTAC	65°C
	Creep700R	CCGCAGTCTGCAGAAAAATG	
P8	Col400F	ACATCGCCCACCACCGGTC	65°C
	Col400R	ACATCGCCCAGAACAGAAAC	

† The numbers preceding the R (Reverse) and F (Forward) refer to the approximate size of the SCAR band in bp. "Creep" indicates marker for creeping bentgrass and "Col" indicates marker for colonial bentgrass.
† Underlined bases indicate the sequence of Operon RAPD primer used.

able band was observed that appropriately indicated creeping bentgrass in Fig. 2a and colonial bentgrass in Fig. 2b. The SCAR primer pair, Creep700F/Creep700R, amplified a single band in only creeping bentgrass plants at approximately 700 bp (Fig. 2a). The SCAR primer pair, Col400F/Col400R, amplified a single band in both colonial and dryland bentgrass species at approximately 450 bp (Fig. 2b). It should be noted that Fig. 2 shows only a small subsample of all 140 DNAs tested. Throughout all of the plants, we did not observe any false positives (amplified bands incorrectly identifying a species).

An explanation for why the Col400F/Col400R SCAR primer pair amplifies in both colonial and dryland bentgrass could be due to their genetic similarities. Creeping bentgrass has a genome constitution of C_2C_2SS, colonial bentgrass has a genome constitution of $C_1C_1C_2C_2$, and velvet bentgrass has a genome constitution of C_1C_1 (Jones, 1955; Wipff and Fricker, 2001). These genome constitutions are diverse enough to create distinct RAPD banding patterns which allow species to be differentiated as can be seen in Fig. 1. However, dryland bentgrass (*A. castellana*) has the same genome constitution as colonial bentgrass ($C_1C_1C_2C_2$) (Jones, 1955). Yamamoto and Duich (1994) also found difficulty in distinguishing colonial and dryland bentgrasses using isozymes. Thus, RAPD banding patterns of colonial and dryland bentgrass are expected to be more similar than other species. The RAPD primers we tested were not sufficiently specific to detect differences between colonial and dryland species; therefore, we could not find a single band to distinguish them. However, we only tested a limited number of RAPD primers, and more primers should be screened for future research.

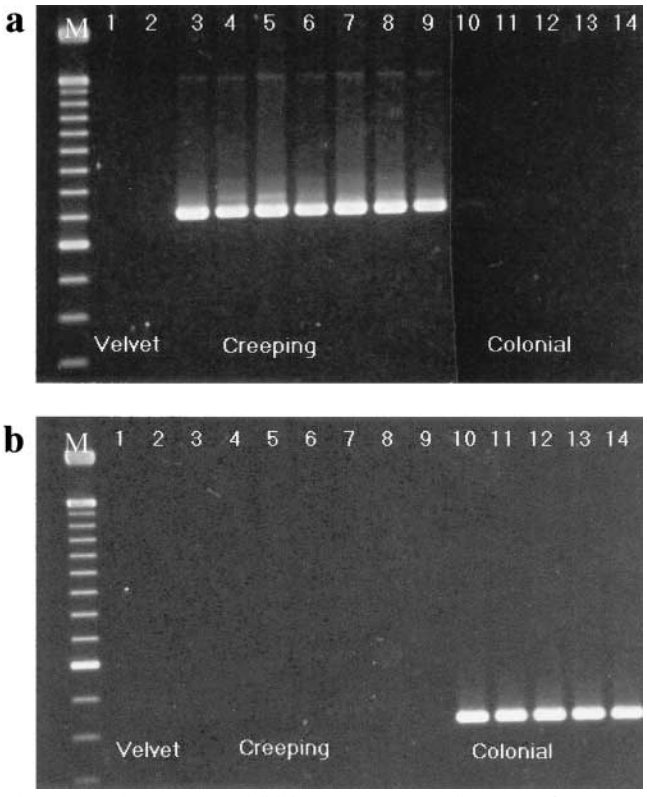

Fig. 2. Banding pattern of SCAR primer pairs Creep700F/R(a) and Col400F/R(b) amplifying a single and reproducible band specifically for creeping and colonial bentgrass species, respectively. Optimal annealing temperature is at 65°C for both sets of SCAR primers. Bentgrass plants indicated are three velvet plants of cultivar EVM 99 (Lanes 1 and 2), 7 creeping plants of Cato (Lanes 3–9), and five colonial plants of 9F7 (Lanes 10–14). *M* indicates 100-bp marker with bright middle band indicating 600 bp.

Creeping and colonial bentgrass are commonly used on golf courses and lawns throughout the temperate world. Both are affected by pathogens such as dollar spot (mainly affects creeping bentgrass), red thread, and brown patch (mainly affects colonial bentgrass). However, colonial bentgrass is naturally more resistant to dollar spot than creeping bentgrass (Meyer and Bonos, 2001). With increasing restrictions on fungicide use, such as the withdrawal of mercury fungicides from the market, and the high cost of fungicide applications, there is a growing need to discover disease resistant clones and incorporate disease resistance into new turfgrass cultivars through breeding. In the USA, most bentgrass cultivars have been bred through collecting candidate clones from golf courses and other established turf where they were challenged by abiotic and biotic stresses over time (Burton, 1992). These challenged clones represent a potential source for disease resistance and other beneficial traits.

The SCAR markers designed in this study can be used for initial screening of bentgrass clones taken from naturalized populations, identifying creeping vs. colonial bentgrasses, and potentially for identifying their natural hybrids. More recently, breeders have been using interspecific hybridization to combine important traits (Burton, 1992; Brilman, 2001a). Hybridizations of different species can be useful for incorporating traits such as disease or drought resistance into new cultivars, making it critically important to identify the hybrids. These designed SCAR markers have potential for identifying and determining which offspring are true hybrids.

Since the number of cultivars used in the development of the SCAR markers was limited, further testing of

more cultivars representing the bentgrass species will be needed. This should be done to reconfirm the validity of the designed SCAR markers. Furthermore, testing of different bentgrass species such as brown bentgrass ($C_1C_1C_2C_2$, *A. vinealis*) (Wipff and Fricker, 2001), may help to find a differentiating DNA marker among them. Once these confirmations are made, further possibilities for maximizing the applications of the SCAR markers could be to perform multiplex PCR with both sets of the designed species-specific SCAR markers. This method would save time and money by running only one reaction per plant sample, instead of several reactions, one for each primer. Preliminary results of multiplex PCR on DNA of known species demonstrated that the SCAR primers could successfully amplify identifying PCR bands under multiplex PCR conditions. To test further possible applications of these SCAR markers, we are currently making crosses of creeping and colonial bentgrass. Once hybrid germplasm is obtained, we will be able to report on the successfulness of these SCAR markers as a tool for hybrid identification.

ACKNOWLEDGMENTS

The authors wish to thank Chris Wendorf of L.L. Olds Seed Co. for providing seed, Jeff Gregos for technical assistance, and Krome Burke-Scoll and Joseph Curley for critical review of the manuscript.

REFERENCES

Beard, J.B. 1973. Turfgrass: Science and culture. Prentice-Hall Inc., Englewood Cliffs, NJ.

Bradshaw, A.D. 1958. Natural hybridization of *Agrostis tenuis* and *Agrostis stolonifera.* New Phytol. 57:66–84.

Brilman, L.A. 2001a. Colonial bentgrass: An option for fairways. Golf Course Manage. 69(1):55–60.

Brilman, L.A. 2001b. Utilization of interspecific crosses for turfgrass improvement. Int. Turfgrass Soc. Res. J. 9:157–161.

Burton, G.W. 1992. Breeding improved turfgrass. p759–775. *In* D.V. Waddington et al. (ed.) Turfgrass. ASA, CSSA, and SSSA, Madison, WI.

Caceres, M.E., F. Pupilli, E. Piano, and S. Arcioni. 2000. RFLP markers are an effective tool for the identification of creeping bentgrass (*Agrostis stolonifera* L.) cultivars. Genet. Res. Crop Evol. 47:455–459.

Dudeck, A.E., and J.M. Duich. 1967. Preliminary investigation on the reproduction and morphological behavior of several selections of colonial bentgrass, *Agrostis tenuis* Sibth. Crop Sci. 7:605–610.

Golembiewski, R.C., T.K. Danneberger, and P.M. Sweeney. 1997. Potential of RAPD markers for use in the identification of creeping bentgrass cultivars. Crop Sci. 37:212–214.

Hernandez, P., A. Martin, and G. Dorado. 1999. Development of SCARs by direct sequencing of RAPD products: a practical tool for the introgression and marker-assisted selection of wheat. Mol. Breed. 5:245–253.

Hitchcock, A.S. 1951. Manual of the grasses of the United States. USDA Misc. Publ. 200. US Gov. Print. Office, Washington, DC.

Huff, D.R., R. Peakall, and P.E. Smouse. 1993. RAPD variation within and among natural populations of outcrossing buffalograss. Theor. Appl. Gen. 86:927–934.

Huff, D.R. 2001. Characterization of Kentucky Bluegrass cultivars using RAPD markers. Int. Turfgrass Soc. Res. J. 9:169–175.

Johns, M.A., P.W. Skroch, J. Nienhuis, P. Hinrichsen, G. Bascur, and C. Munoz-Schick. 1997. Gene pool classification of common bean landraces from Chile based on RAPD and morphological data. Crop Sci. 37:605–613.

Jones, K. 1955. Species differentiation in *Agrostis.* J. Genet. 54(3): 370–376.

Jones, T.W.A. 1983. Instability during storage of phosphogluco-isomerase isoenzymes from ryegrasses (*Lolium* spp.). Physiol. Plant 58:136–140.

Jung, G., P.W. Skroch, J. Nienhuis, D.P. Coyne, E. Arnaud-Santana, H.M. Ariyarathne, and J.M. Marita. 1999. Confirmation of QTL associated with common bacterial blight resistance in four different genetic backgrounds in common bean. Crop Sci. 39:1448–1455.

Meyer, W.A., and S.A. Bonos. 2001. Trends in cool-season turfgrass breeding. Golf Course Manage. 69(9):61–64.

Murphy, J.A., W.A. Meyer, K.A. Plumley, B.B. Clarke, S.A. Bonos, W.K. Dickson, J.B. Clark, D.A. Smith, and T.J. Lawson. 2000. Performance of bentgrass cultivars and selections in New Jersey turf trials. *In* 1999 Rutgers Turfgrass Proceedings. New Jersey Turfgrass Expo, Atlantic City, NJ.

Paran, I., and R.W. Michelmore. 1993. Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. Theor. Appl. Gen. 85:985–993.

Skroch, P., and J. Nienhuis. 1995. Impact of scoring error and reproducibility of RAPD data on RAPD based estimates of genetic distance. Theor. Appl. Gen. 91:1086–1091.

Stuckey, A.H., and W.G. Banfield. 1946. Morphological variations and the occurance of aneuploids in some species of *Agrostis* in Rhode Island. Am. J. Bot. 33:185–190.

Vidal, J.R., P. Delavault, M. Coarer, and A. Defontaine. 2000. Design of grapevine (*Vitas vinifera* L.) cultivar-specific SCAR primers for PCR fingerprinting. Theor. Appl. Gen. 101:1194–1201.

Wang, Z., M.D. Casler, J.C. Stier, J. Gregos, S.M. Millet, and D.P. Maxwell. 2000. Speckled snow mold resistance in creeping bentgrass. p. 151. *In* Agronomy Abstracts. ASA, Madison, WI.

Warnke, S.E., D.S. Douches, and B.E. Branham. 1997. Relationships among creeping bentgrass cultivars based on isozyme polymorphisms. Crop Sci. 37:203–207.

Wilkinson, J.F., and J.B. Beard. 1972. Electrophoretic identification of *Agrostis palustris* and *Poa pratensis* cultivars. Crop Sci. 12:833–834.

Wipff, J.K., and C. Fricker. 2001. Gene flow from transgenic creeping bentgrass (*Agrostis stolonifera* L.) in the Willamette Valley. Oregon. Int. Turfgrass Soc. Res. J. 9:224–242.

Yamamoto, I., and J.M. Duich. 1994. Electrophoretic identification of cross-pollinated bentgrass species and cultivars. Crop Sci. 34: 792–798.